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A Lignified Star-Shape Cavity at Root-Bole Interface: An Appropriate Culture Chamber for *Ganoderma boninense* and Stromatic-Like Structure Development[1]

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ABSTRACT

This study provides further information on the role of the melanised stromatic-like structure (SLS) for the initiation of Basal Stem Rot (BSR) infection in both the field and nursery. Artificial inoculation of seedlings by using Ganoderma*-colonized rubber wood block (RWB) previously cleaned of SLS confirmed the essential role of this structure to induce rapid and homogeneous infections. In mature palms, whatever the sanitary condition, the presence of a highly lignified star-shape cavity at the base of the bole (root-bole interface) could play a role as a culture chamber for* Ganoderma *development and infection. This cavity is considered to provide suitable conditions (dark, lower than ambient temperature, high humidity and a dense substrate) for the development of SLS and probably has less antagonists than in the open soil where* Ganoderma *is a poor competitor. A lignified scar which will develop as a future cavity has been observed in eight month-old nursery seedlings. Field observations of infected palms confirm the presence of a melanised fungal tissue inside this lignified star-shape cavity. Random observations of the initial stage of infection of young palms revealed that the development of SLS inside the cavity occurred before the penetration and colonisation of the oil palm bole. Transverse and longitudinal sections of the root-bole interface from recently infected palms seem to demonstrate that the initial point of* Ganoderma *penetration was confined to this lignified cavity. No initial* Ganoderma *penetration points were observed beyond the periphery of this star-shape cavity. The colonisation of the bole by the fungus shows a centrifugal and radial invasion from the cavity which corroborates the important role of this lignified cavity for* Ganoderma *penetration. These results do not exclude multiple infections from this cavity by more than one isolate. The common point between field observations of mature palm infection and a nursery inoculation tests is the prior development of SLS on a dense substrate (lignified cavity in field and RWB in nursery). The sequence of events concerning the mature bole invasion by* Ganoderma *proposed in this paper is discussed.*

Key words: Elaeis guineensis*, oil palm,* Ganoderma boninense*, basal stem rot, stromatic-like structure, pseudo-sclerotium, rhizotron, lignified star-shape cavity*

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INTRODUCTION

Basal Stem Rot (BSR) of oil palm (*Elaeis guineensis* Jacq.) is caused by *Ganoderma boninense*, which is one of the most devastating diseases in South-East Asia. Some questions remain unresolved concerning the spread of the disease by basidiospores or root contact infection. A large number of studies have demonstrated successful infection by root contact with an inoculum source (Navaratnam and Chee, 1965; Sariah *et al*., 1994; Hasan & Turner, 1998; Breton *et al*., 2005a) and support the view that root infection is the primary pathway for the development of BSR disease. In this context, the current study was set up to continue our investigations of the important role of melanised stromatic-like structure (SLS) produced by *Ganoderma* and to bring further evidence of its implication in the bole invasion of mature palms within plantations. The development of SLS or pseudo-sclerotium produced by *Ganoderma* was described by Breton *et al.* (2005b) and was confirmed more recently by Rees *et al.* (2009). The monitoring of *Ganoderma* infection by a rhizotron method (or glass chamber) demonstrated a coating by this fungal hardened tissue on some roots in direct contact with the *Ganoderma* inoculated RWBs. This strictly close contact was associated with the point of primary penetration of the pathogen in intact roots (Breton *et al*., 2005b). The development of this compact mass of hardened mycelium is only observed on a dense substrate such as rubber wood block but not on rubber wood sawdust. Results from rhizotron and nursery trials demonstrated clearly that SLS development was necessary for successful and homogeneous infection of seedlings (Breton *et al*., 2005b; 2006).

MATERIALS & METHODS

Mature Palms Observations

A Random sample of infected mature palms within plantations of a range of planting years were excavated, then longitudinal and transverse sections of the root-bole interface were performed.

Nursery inoculation

Ganoderma-colonized RWBs obtained after several weeks of incubation in the dark at 27°C were used as the inoculum source. The planting material came from the seed production sections of Lonsum's Sumatra Bioscience and Socfindo's Bangun Bandar Estate. An artificial oil palm germinated seed inoculation method, described by Breton *et al.* (2005a) for *Ganoderma* screening, was used to obtain rapid and homogeneous disease symptoms. Melanised SLS was discarded from RWB surfaces by using a scalpel to obtain SLS-free *Ganoderma*-colonized RWB. The distance between the upper face of the RWB to the germinated seeds was standardised.

Rhizotron or glass chamber method

Inoculation of germinated seeds in rhizotron (constituted of two plates of glasses separated by three cm) was performed with RWBs previously colonized by *Ganoderma*. Rhizotrons were inoculated and incubated according to the method of Breton *et al*. (2005b), then the infection process was monitored and recorded on a weekly basis.

RESULTS & DISCUSSION

The rhizotron method showed melanised SLS at the surface of the inoculum source surrounding the roots in contact with the RWB (Fig. 1). The kinetics of the symptoms appearance (external and internal) demonstrate that this close contact is the first step for *Ganoderma* root penetration and colonisation. Breton *et al.* (2005a) demonstrated by rhizotron observations that the development of the SLS occurred only on the dense substrate and that the presence of this melanised mycelium mass is necessary to induce BSR symptoms. Nursery inoculation of germinated seed using *Ganoderma*-colonized RWB confirmed that the presence of SLS on the inoculum surface is an important factor for the seedlings infection (Fig. 2). The infection observed for the treatment SLS-free RWB correspond to a production *de novo* of the SLS. Observations of the RWBs at this end of this trial confirmed this hypothesis of a continuous production. Less than 2% of infected seedlings were obtained by using SLS alone and no SLS was found in the soil at this end of the trial (Fig. 2). These results demonstrated that this fungal melanised mass need a hardened food base to survive in the soil to express its high infectious potential.

In natural conditions of infection, the melanised SLS were observed especially at the external surface of a lignified star-shape cavity localised at the base of the stem, root-bole interface (Fig. 3a-b). This cavity or crack, common to all palms whatever their sanitary conditions, has probably originated as a physical phenomena related to the natural oil palm growth (Fig. 3c-d). Randomised observations of this lignified cavity on healthy palms, show the characteristics of this scar could play a role as a culture chamber for *Ganoderma* development (SLS production) because it is dark, humid, lower than ambient temperatures, dense substrate and also probably has less *Ganoderma* competitors than in the open soil.

Rotten cracks frequently observed on the basal stems of highly or medium infected mature palms are always observed as a centrifugal degradation of the bole tissues (from inside to outside). This internal progression of fungus invasion can also be visualised at the base of the stem from medium infected palm from the colour of rotten tissues (Fig. 4a). Transverse and longitudinal sections of the root-bole interface from recently infected young palm confirmed a centrifugal and radial initial invasion of the bole from this lignified cavity (Fig. 4b-c). If no initial penetration points were found beyond the periphery of this lignified area, multiple penetration points were observed from this cavity which probably involved more than one isolate. Moreover, in the case of advanced stages (rotten bole tissues) of *Ganoderma* invasion, several points of fungus penetration inside the bole were observed at the peripheral of the basal stem. These observations demonstrate the importance of this lignified cavity as an efficient dense substrate for the SLS production prior to the invasion of the bole (early stage of infection). Rees *et al.* (2009) described in advanced stages of *Ganoderma* infection multiple peripheral invasions in the bole. The observations of the authors are not in contrast with the present work. In this study, random observations of initial invasion were realized by longitudinal and transverse sequential sections inside the bole from recently infected young palms, which permitted the localisation of the initial penetration area (Fig. 4b-c). In parallel, it is important to notify, that this lignified cavity was not always observed in central position at the basal stem (root-bole interface).

At the nursery stage, a lignified scar, prelude to the formation of this cavity on mature palms, could be clearly observed on eight months-old seedling (Fig. 5a). Recent observations implemented through a large number of dissection experiments showed this lignified scar on five months-old seedlings (Jacquemard, personal communication). According to the age of the seedling, the size and the depth of this scar could be sufficient for *Ganoderma* to use it as a substrate for SLS production (Fig. 5b). In the nursery screening test using germinated seed, the kinetics of the first symptoms appear 10 weeks after inoculation and long before the appearance of the scar at around 5 months

after nursery planting of germinated seed. This demonstrates that the scar is not the entry point for *Ganoderma* infection in nursery screening trials. In nursery screening trials the RWB provides a dense substrate to reproduce or simulate the role of the lignified star-shape cavity observed in mature palms. The melanised SLS, produced by *Ganoderma* only on dense substrates and essential to infection, could be considered as an aggressive or pathogenic stage in the physiological life cycle of the fungus. Sections of *Ganoderma*-colonized RWB performed after different incubation times demonstrate that the production of SLS at the RWB surface was the first step before internal invasion of the RWB by the fungus. This result corroborates the important role of the melanised SLS in colonising host tissues.

CONCLUSION

Stromatic-like structure (SLS) or pseudo-sclerotium produced by *Ganoderma* seems to be an essential physiological stage prior the invasion of the oil palm bole. For mature palms, the presence of a lignified star-shape cavity at root-bole interface permits the development of SLS prior colonization of the basal stem. Same issue was demonstrated by using *Ganoderma* colonized-RWB as inoculum source to infected seedlings. In artificial inoculation, RWB as substrate source (dense substrate) simulate the role of the lignified cavity at root-bole interface. The presence of this cavity which plays the role of *Ganoderma* culture chamber for SLS development appears to be the major location for the initial invasion of the fungus inside the bole. There is variation in the size and depth of the lignified star-like cavity. Insufficient field observations have been conducted to establish any relationship between cavity size with genetic origin of oil palms or environmental factors (soil characteristics). Work is in progress to investigate the relationship between the size of the basal lignified star-like cavity with the resistance/susceptibility of oil palm or with the kinetic of the bole invasion by *Ganoderma*. In parallel, localised injections (fungicides and antagonists microorganisms) in and around this lignified star-shape cavity are being tested

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Figure 1: Macroscopic observations after germinated seeds inoculation using rhizotron method. The stromatic-like structures (SLS) envelop the roots (arrows) prior the induction of external and internal disease symptoms. This close contact root-SLS is the preliminary step of the internal invasion.

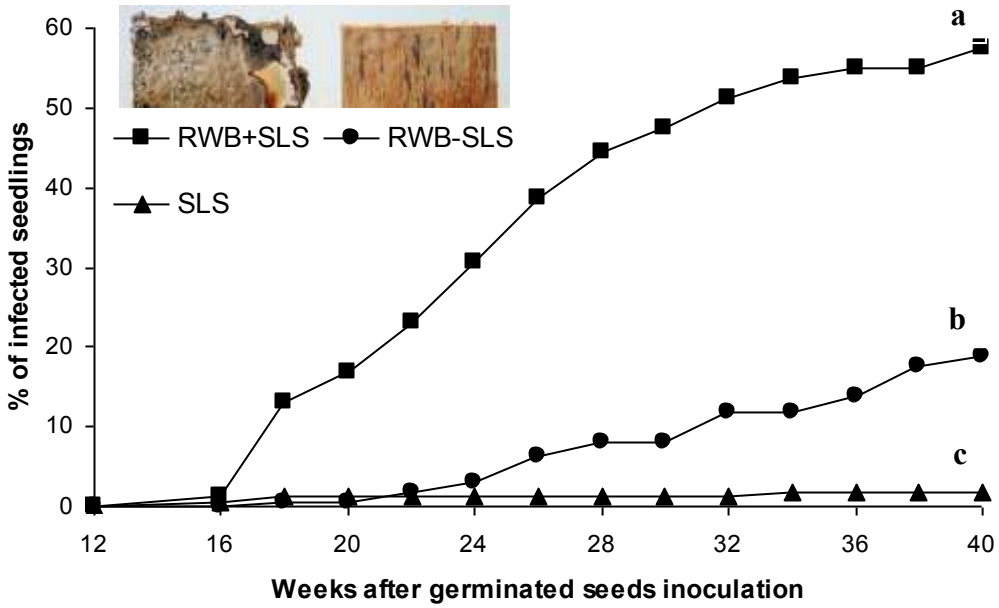

Figure 2: Kinetics of BSR development following artificial inoculation of oil palm germinated seeds by *Ganoderma boninense*. Three kind of inoculum sources were tested; standard *Ganoderma*-colonized RWB (RWB+SLS), free SLS *Ganoderma*-colonized RWB (RWB-SLS) and stromatic-like structure alone (SLS). Each data point represents the average of a total of 8 replicates of 20 seedlings. Curves with a common letter are not statistically significant by Tukey test at p=0.005.

Figure 3: Field observations of the lignified star-shape cavity present at root-bole interface from mature palms planted at Indonesia (a,b: infected palm, c: healthy palm) and at Equador (d). A remaining fragment of the lignified cavity from infected palm (b) revealed the presence of stromatic-like structures (arrow) covering the surface.

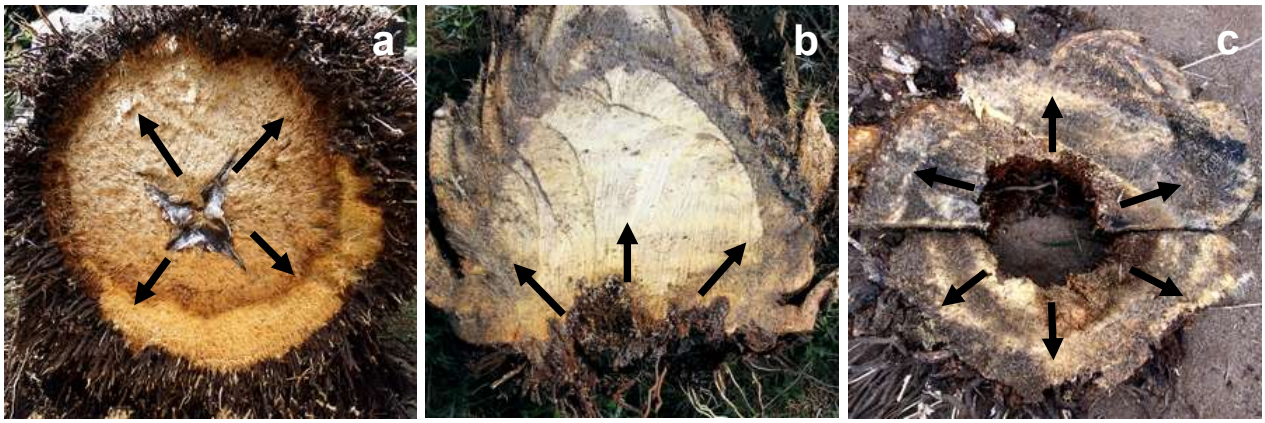

Figure 4: Observation of root-bole interfaces from recently infected mature palms within plantations. Photos (b) and (c) are longitudinal and transverse section of this zone respectively. Arrows show the progression of the rotten tissues from the basal lignified cavity indicating a radial and a centrifugal colonisation of the bole by *Ganoderma*.

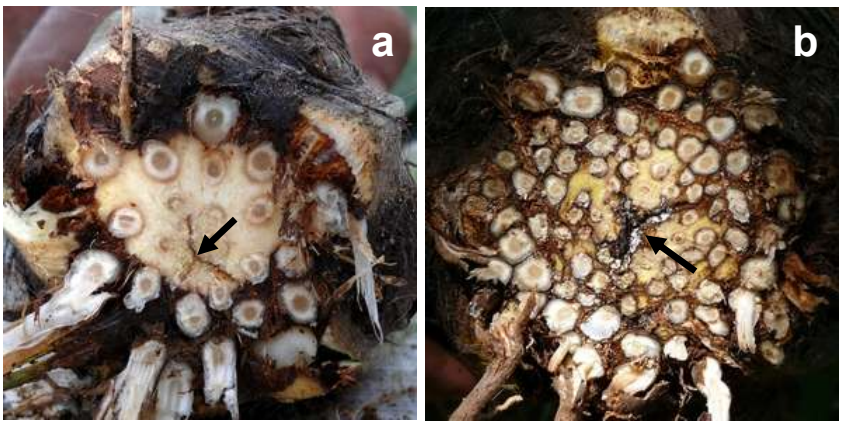

Figure 5: Macroscopic observation of the root-bole interface in eight (a) and eighteen (b) months-old seedlings. Arrows indicate the lignified scar which will form the future star-shape cavity observed in mature palms.